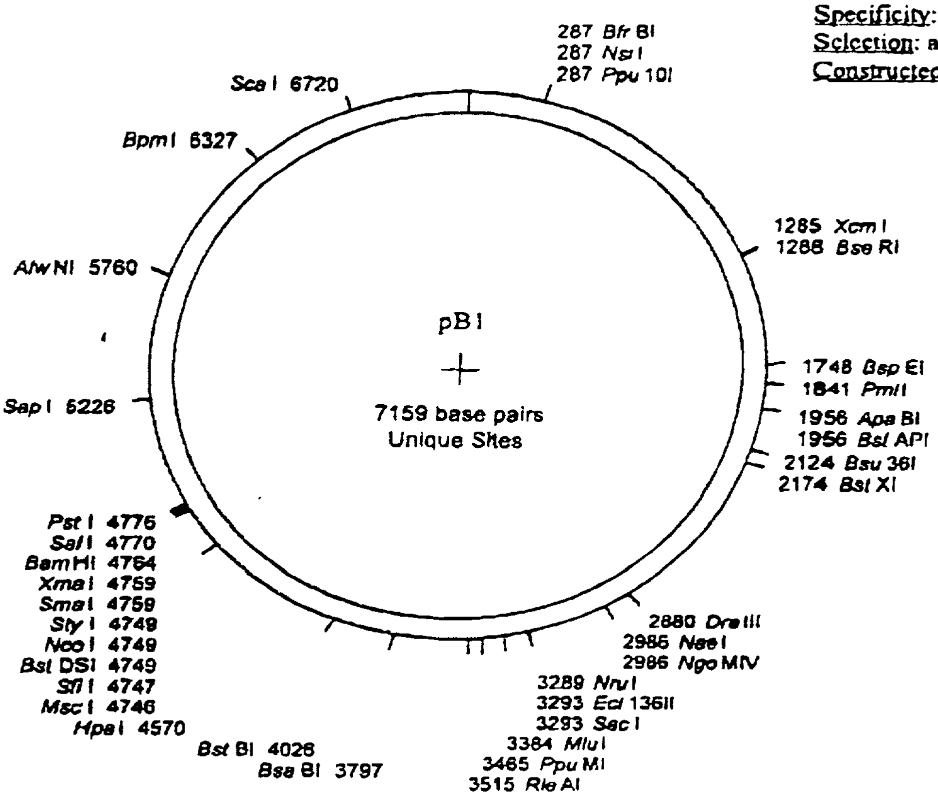
pB1

Alias: pAS2DD
Application: 2HY (bait)
Backbone:
Specificity:
Selection: ampicillin
Constructed by:



Oligo 160

gagagtagtaacaaaggtc AAAGACAGTTGACTGTATCGCCG GAA TTT AT

Sfi I — Sma I — BamH I — Sal I — Pst I

G GCC ATG GAG GCC CCG GGG ATC CGT CGA CCT GCA GCC

Nco I

Oligo 161

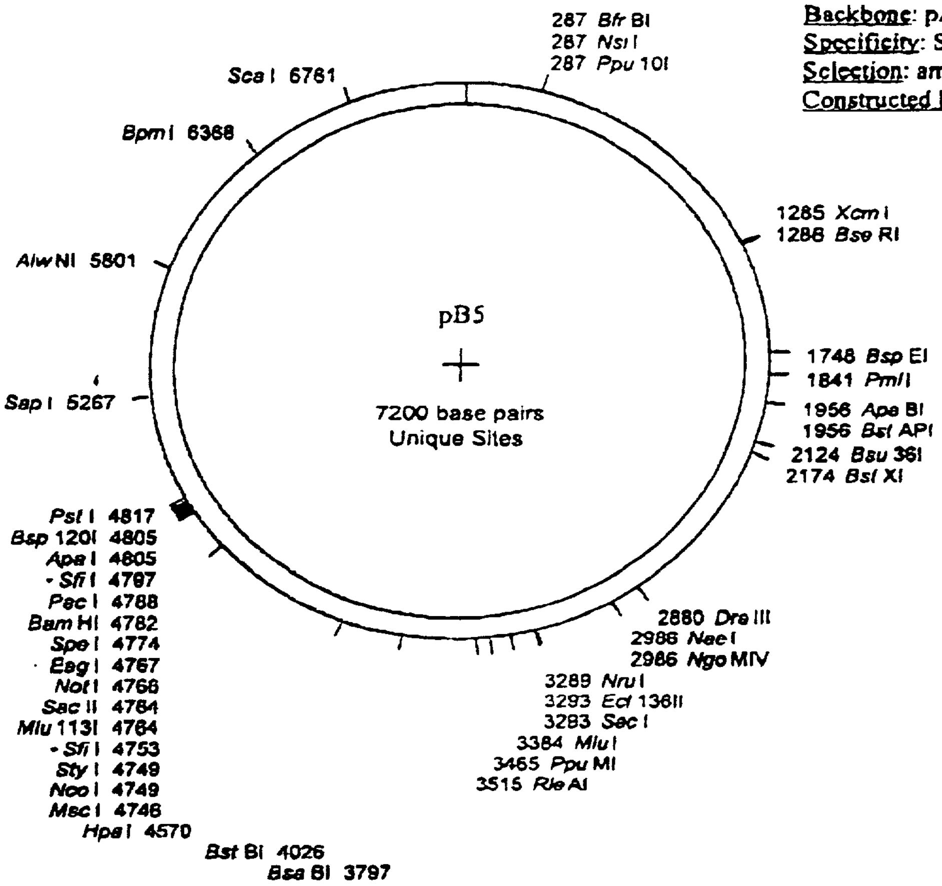
AAG CTA ATT ccgggcgaatttcttatg

Oligo 160 5' GAGAGTAGTAACAAAGGTC 3'
Oligo 161 5' CATAAGAAATTCGCCCGG 3'

FIGURE 1

pB5

Alias: pAS2DDNS1
Application: 2HY (bait)
Backbone: pAS2DD
Specificity: Sfi non-oriented
Selection: ampicillin
Constructed by: SW



Oligo 160

gagagtagtaacaaaggtc AAAGACAGTTGACTGTATCGCCG GAA TTT ATG

Sfi I — Sac II — Spe I — Bam HI

GCC ATG GCC GCA GGG GCC GCG GCC GCA CTA GTG GGG ATC C

Nco I — Not I

STOP — Sfi I — Pst I

TT AAT TAA GGG CCA CTG GGG CCC CTC GAC CTG CAG CCA

Pac I

Oligo 161

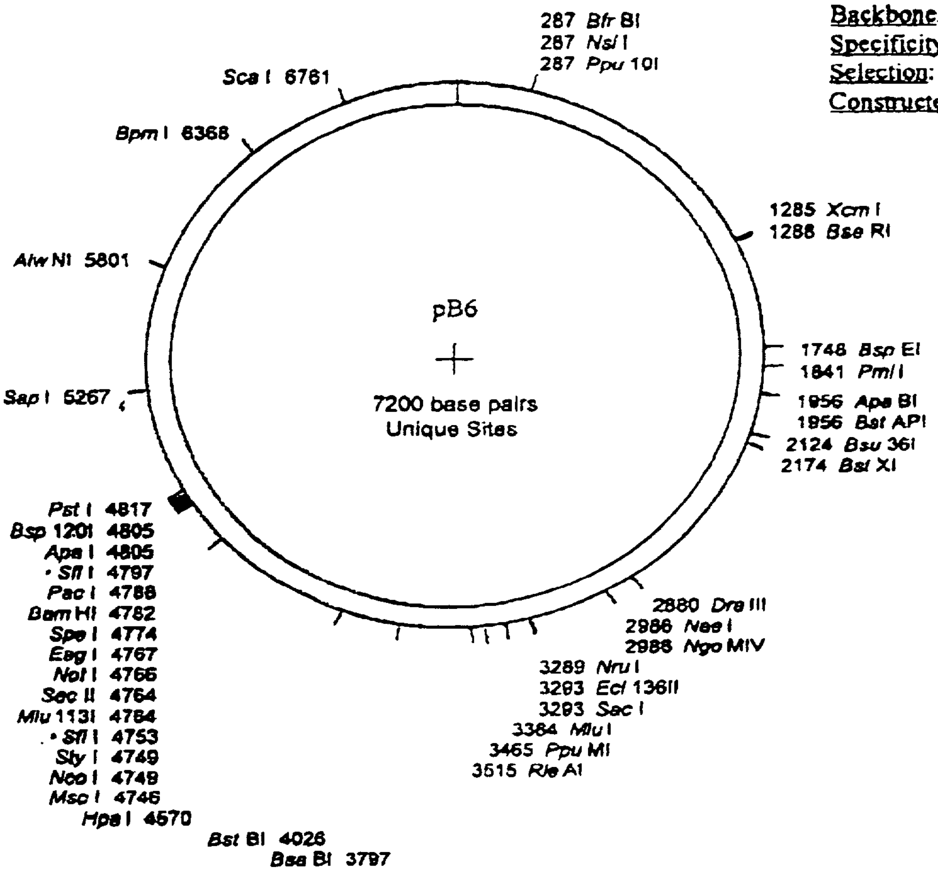
AGC TAA TT ccgggcgaatttcttatg

Oligo 160 5' GAGAGTAGTAACAAAGGTC 3'
Oligo 161 5' CATAAGAAATTCGCCCGG 3'

FIGURE 2

pB6

<u>Application</u>: 2HY (bait)
<u>Backbone</u>: pAS2DD
<u>Specificity</u>: Sfi oriented
<u>Selection</u>: ampicillin
<u>Constructed by</u>: SW



Oligo 160

gagagtagtaacaaaggtc AAAGACAGTTGACTGTATCGCCG GAA TTT ATG

Sfi I **Sac II** **Spe I** **Bam HI**

GCC ATG GCC GGA CGG GCC GCG GCC GCA CTA GTG GGG ATC C

Nco I **Not I**

STOP **Sfi I** **Apa I** **Pst I**

TT AAT TAA GGG CCA CTG GGG CCC CTC GAC CTG CAG CCA

Pac I

Oligo 161

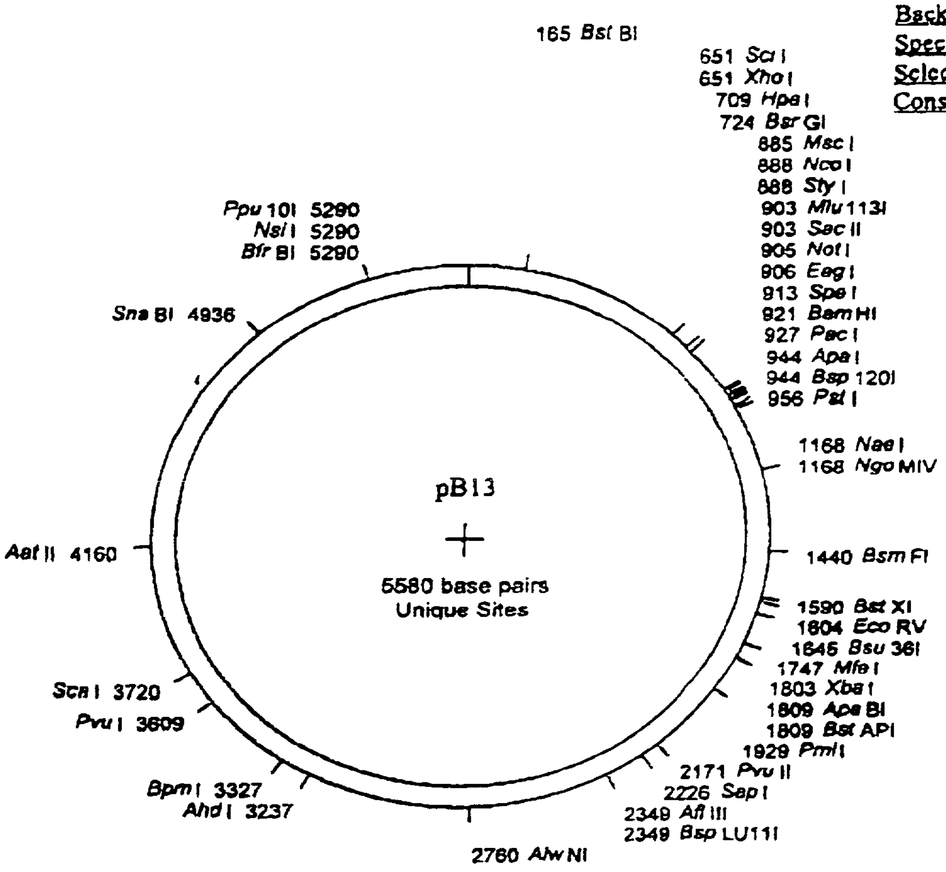
AGC TAA TT ccgggcgaatttcttatg

Oligo 160 5' GAGAGTAGTAACAAAGGTC 3'
Oligo 161 5' CATAAGAAATTCGCCCGG 3'

FIGURE 3

pB13

Alias: pGBT9NSI
Application: 2HY (bait)
Backbone: pGBT9
Specificity: Sfi non-oriented
Selection: ampicillin
Constructed by: CR



Oligo 160

gagagtagtaacaaaggtc AAAGACAGTTGACTGTATCGCCG GAA TTT ATG

Sfi I **Sac II** **Spe I** **Bam HI**

GCC ATG GCC GCA GGG GCC GCG GCC GCA CTA GTG GGG ATC C

Nco I **Not I**

STOP **Sfi I** **Pst I**

TT AAT TAA GGG CCA CTG GGG CCC CTC GAC CTG CAG CCA

Pac I

Oligo 161

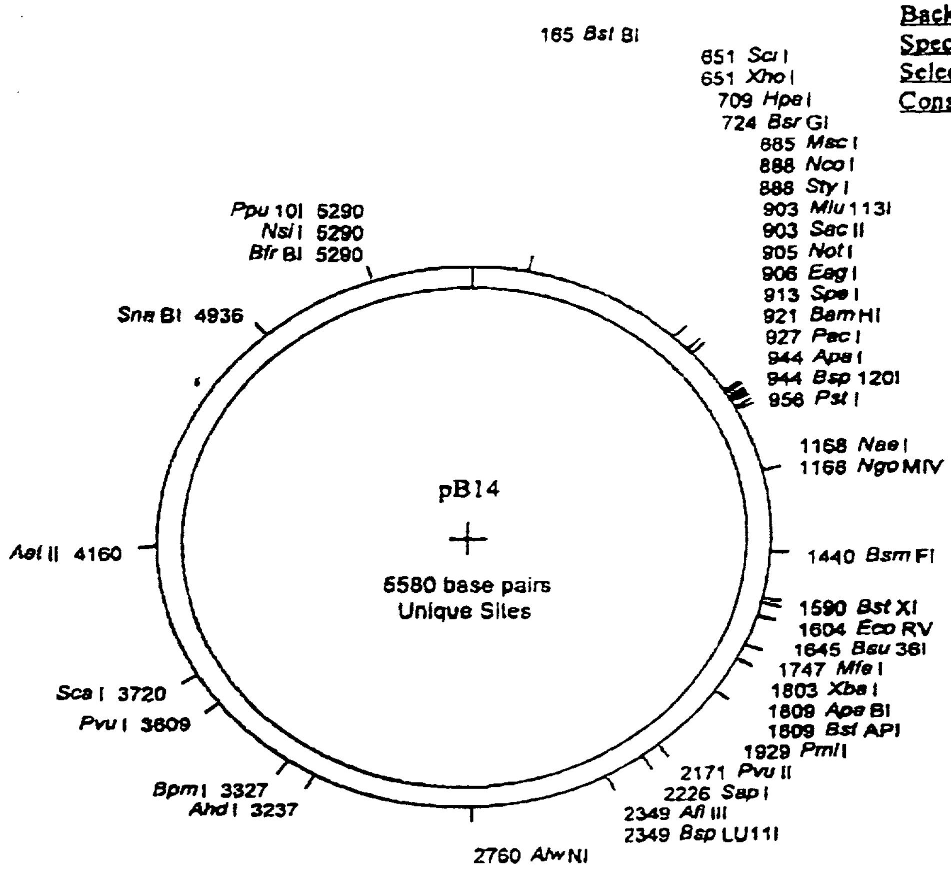
AGC TAA TT ccgggcgaatttcttatg

Oligo 160 5' GAGAGTAGTAACAAAGGTC 3'
Oligo 161 5' CATAAGAAATTCGCCCGG 3'

FIGURE 4

pB14

<u>Alias</u>: pGBT9NS2
<u>Application</u>: 2HY (bait)
<u>Backbone</u>: pGBT9
<u>Specificity</u>: Sfi oriented
<u>Selection</u>: ampicillin
<u>Constructed by</u>: CR



Oligo 160

gagagtagtaacaaaggtc AAAGACAGTTGACTGTATCGCCG GAA TTT ATG

Sfi I — Sac II — Spe I — Bam HI

GCC ATG GCC GGA CGG GCC GCG GCC GCA CTA GTG GGG ATC C

Nco I — Not I

STOP — Sfi I — Apa I — Pst I

TT AAT TAA GGG CCA CTG GGG CCC CTC GAC CTG CAG CCA

Pac I

Oligo 161

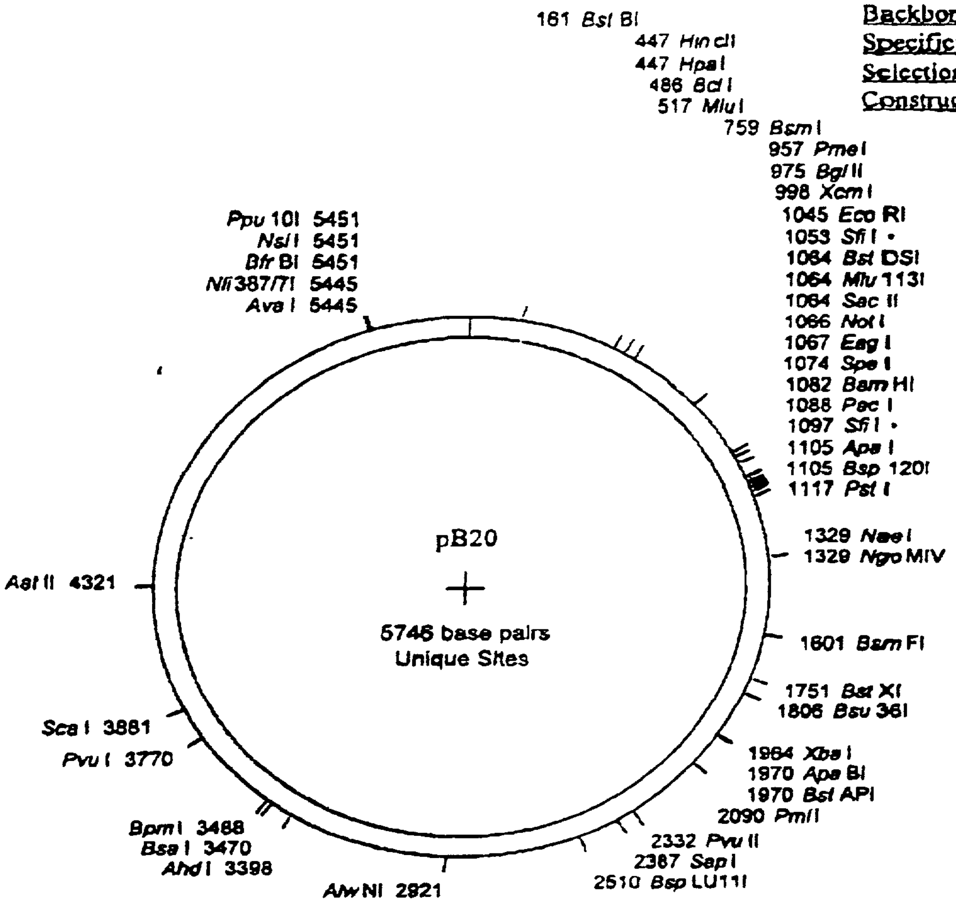
AGC TAA TT ccgggcgaatttcttatg

Oligo 160 5' **GAGAGTAGTAACAAAGGTC** 3'
Oligo 161 5' **CATAAGAAATTCGCCCGG** 3'

FIGURE 5

pB20

Alias: pLex10NS2
Application: 2HY (bait)
Backbone: pLex10 (pB9)
Specificity: Sfi-oriented
Selection: ampicillin
Constructed by: LD



EcoR I **Sfi I** **Not I** **Spe I** **BamH I**

GAA TTC GGG GCC GGA CGG GCC GCG GCC GCA CTA GTG GGG ATC C

Sac II

STOP

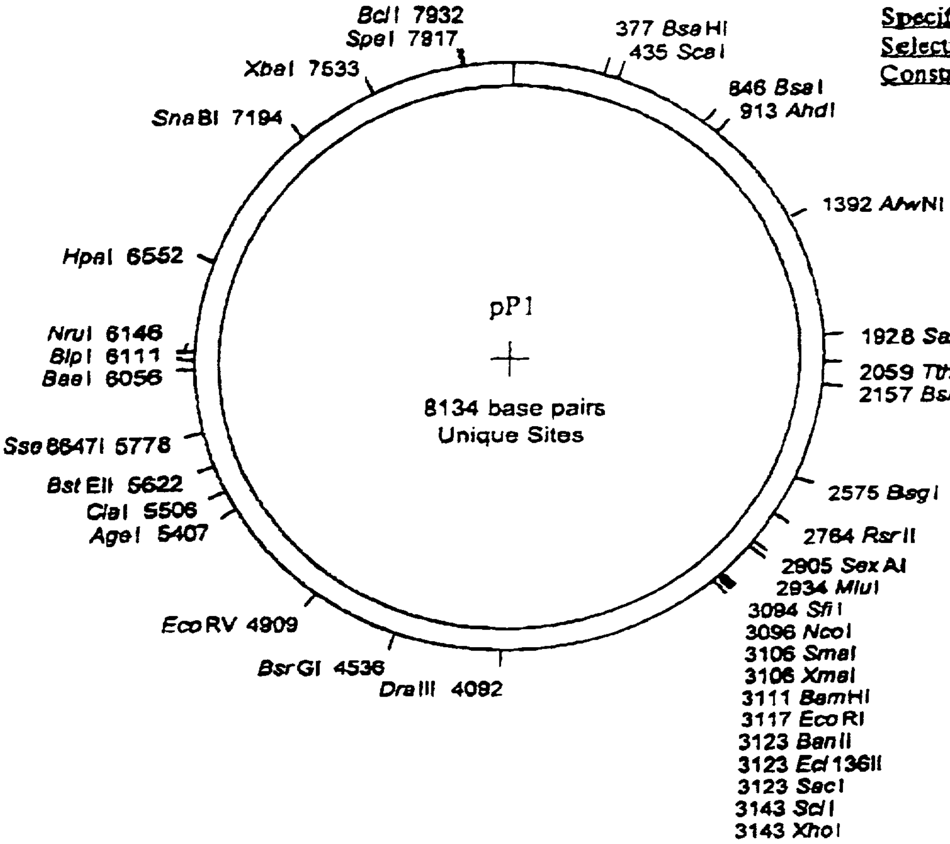
TT AAT TAA GGG CCA CTG GGG CCC CTC GAC CTG CAG

Pac I **Sfi I** **Pst I**

FIGURE 6

pP1

<u>Alias</u> : pACTIIst
<u>Application</u>: 2HY (prey)
<u>Backbone</u>: pACTII
<u>Specificity</u>:
<u>Selection</u>: ampicillin
<u>Constructed by</u>:



ABS1
cgtttggaatcactacagg GATGTTTAATACCACTACAATGGATGATGTATATAACTATCTATT

JC90 **Bgl II**
cgatgatgaagataccccaccaaa CCCAAAAAAAGAGATCTGTATGGCTTACCCATACGATGTTCCAG

Sfi I **Sma I** **BamH I**
ATTACGCTAGCTTGGGTGGTCATATGGCC ATG GAG GCC CCG GGG ATC CGA ATT
Nco I

Sac I **Xho I** **Bgl II**
CGA GCT CGA CTA GCT AGC TGA CTC GAG AGA TCT ATGAAT

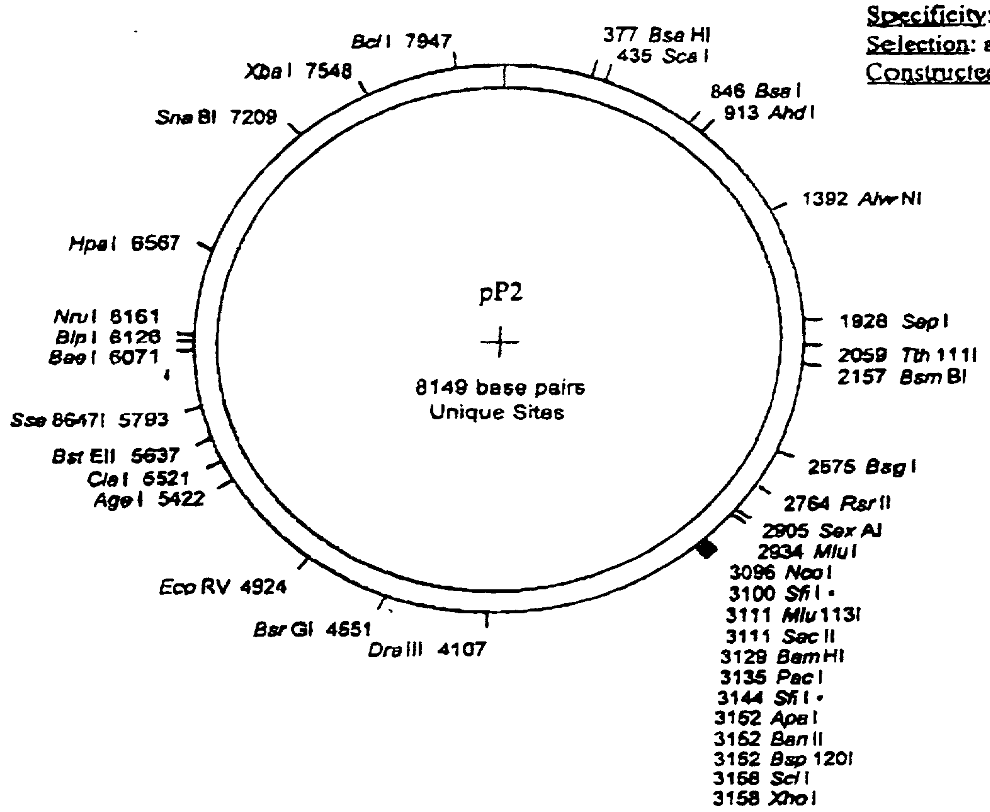
cgtagatactgaaaaacccc GCAAGTT cacttcaactgtgcatcgtg caccatctcaatttc
162 **ABS2** **53**

ABS1 5' CGTTTGGAATCACTACAGG 3'
JC90 5' CGATGATGAAGATACCCCACCAAA 3'
162 5' GGGGTTTTTCAGTATCTACG 3'
ABS2 5' CACGATGCACAGTTGAAGTG 3'
53 5' GAAATTGAGATGGTGCACGATGCAC 3'

FIGURE 7

pP2

<u>Application</u>: 2HY (prey)
<u>Backbone</u>: pACTIIst
<u>Specificity</u>:Sfi non-oriented
<u>Selection</u>: ampicillin
<u>Constructed by</u>:SW



ABS1
CG cgtttggaatcactacagg GATGTTTAATACCACTACAATGGATGATGTATATAACTATCTATT

JC90 **Bgl II**
cgatgatgaagataccccaccaaa CCCAAAAAAAGAGATCTGTATGGCTTACCCATACGATGTTCCAG

Sfi I **Sac II**
ATTACGCTAGCTTGGGTGGTCATATGGCC ATG GCC GCA GGG GCC GCG GCC GCA
Nco I

BamH I **Pac I**
CTA GTG GGG ATC CTT AAT TAA GGG CCA CTG GGG CCC CTC GAG AGA TCT
Stop

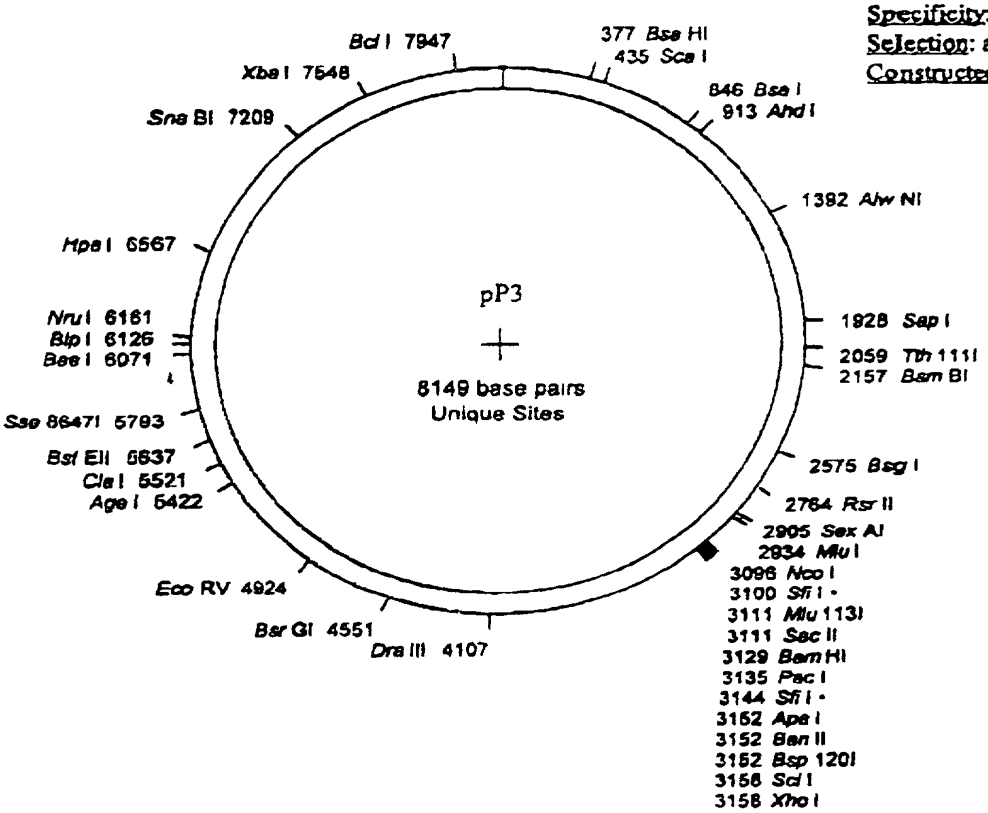
ATGAAT cgtagatactgaaaaacccc GCAAGTT cacttcaactgtgcatcgtg caccatctcaatttc
162 ABS2 53

ABS1 5' **CGTTTGGAATCACTACAGG** 3'
JC90 5' **CGATGATGAAGATACCCCACCAAA** 3'
162 5' **GGGGTTTTTCAGTATCTACG** 3'
ABS2 5' **CACGATGCACAGTTGAAGTG** 3'
53 5' **GAAATTGAGATGGTGCACGATGCAC** 3'

FIGURE 8

pP3

Application: 2HY (prey)
Backbone: pACTIIst
Specificity: Sfi oriented
Selection: ampicillin
Constructed by: SW



ABS1
CG cgtttggaatcactacagg GATGTTTAATACCACTACAATGGATGATGTATATAACTATCTATT

JC90 Bgl II
cgatgatgaagataccccaccaaa CCCAAAAAAAGAGATCTGTATGGCTTACCCATACGATGTTCCAG

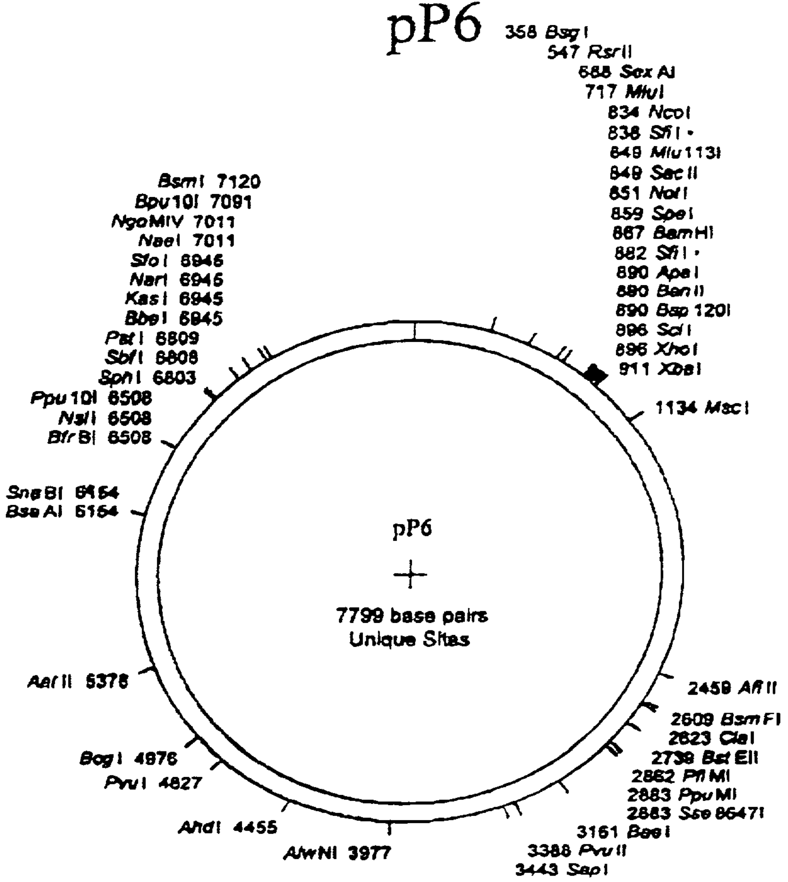
Sfi I Sac II
ATTACGCTAGCTTGGGTGGTCATATGGCC ATG GCC GGA CGG GCC GCG GCC GCA
Nco I

BamH I Pac I
CTA GTG GGG ATC CTT AAT TAA GGG CCA CTG GGG CCC CTC GAG AGA TCT
Stop

ATGAAT cgtagatactgaaaaacccc GCAAGTT cacttcaactgtgcatcgtg caccatctcaatttc
162 ABS2 53

ABS1 **5' CGTTTGGAATCACTACAGG 3'**
JC90 **5' CGATGATGAAGATACCCCACCAAA 3'**
162 **5' GGGGTTTTTCAGTATCTACG 3'**
ABS2 **5' CACGATGCACAGTTGAAGTG 3'**
53 **5' GAAATTGAGATGGTGCACGATGCAC 3'**

FIGURE 9



Alias: pGAD3S2XNS1
Application: 2HY (prey)
Backbone: pGAD3S2X
Specificity: Sfi non-oriented
Selection: ampicillin
Constructed by: SW

ABS1

cgtttggaatcactacagg GATGTTTAATACCACTACAATGGATGATGTATATAACTATCTATT

JC90

cgatgatgaagataccccaccaaa CCCAAAAAAAGAGATCCTAGAACTA

Sfi I **Sac II** **Spe I** **Bam HI**

GCC ATG GCC GCA GGG GCC GCG GCC GCA CTA GTG GGG ATC C

Nco I **Not I**

STOP **Sfi I** **Xbo I** **Xba I**

TT AAT TAA GGG CCA CTG GGG CCC CTC GAG TAG CTA GTG TCT AGA

STOP **STOP** **STOP**

GGCCCGGTACCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCG TCGTTTTA

CAACGTCGTGACTGGGAAAACCCTGATCTATGAAT cgtagatactgaaaaacccc GCAA

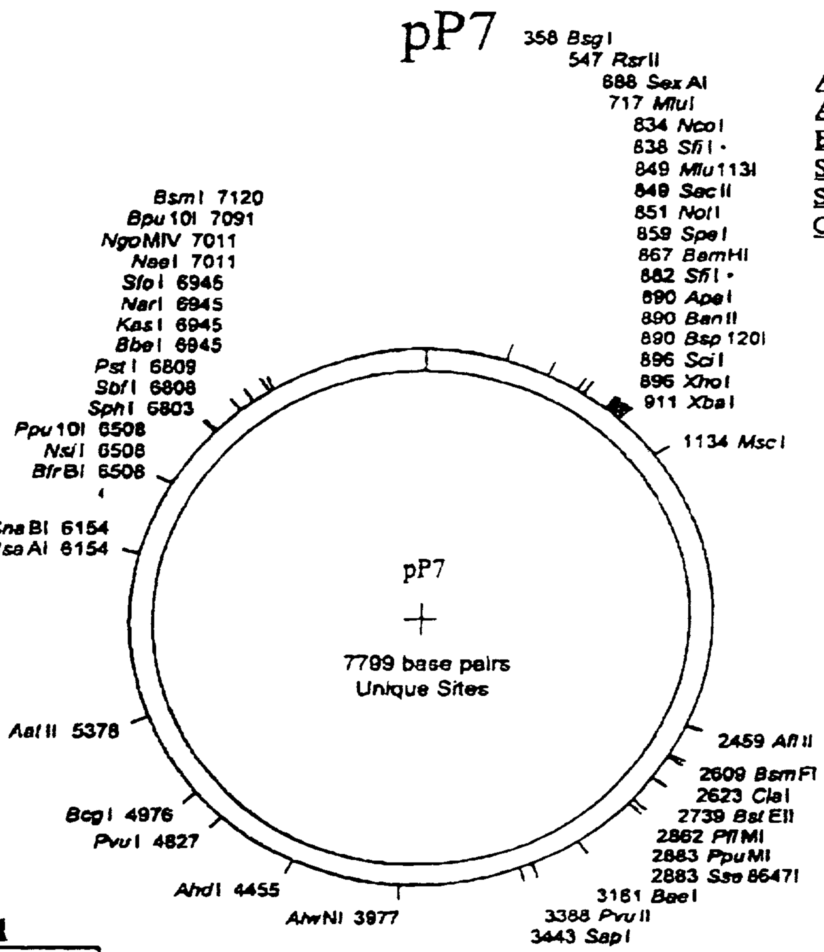
162

GTT cacttcaactgtgcatcgtg caccatctcaatttcttc

ABS2 **53**

ABS1 5' CGTTTGGAATCACTACAGG 3'
JC90 5' CGATGATGAAGATACCCCACCAAA 3'
162 5' GGGGTTTTTCAGTATCTACG 3'
ABS2 5' CACGATGCACAGTTGAAGTG 3'
53 5' GAAATTGAGATGGTGCACGATGCAC 3'

FIGURE 10



Alias: pGAD3S2XNS2
Application: 2HY (prey)
Backbone: pGAD3S2X
Specificity: Sfi oriented
Selection: ampicillin
Constructed by: SW

ABS1
cgtttggaatcactacagg GATGTTTAATACCACTACAATGGATGATGTATATAACTATCTATT

JC90
cgatgatgaagataccccaccaaa CCCAAAAAAAGAGATCCTAGAACTA

Sfi I **Sac II** **Spe I** **Bam HI**
GCC ATG GCC GGA CGG GCC GCG GCC GCA CTA GTG GGG ATC C
Nco I **Not I**

STOP **Sfi I** **Xho I** **Xba I**
TT AAT TAA GGG CCA CTG GGG CCC CTC GAG TAG CTA GTG TCT AGA
STOP **STOP** **STOP**

GGCCCGGTACCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTA

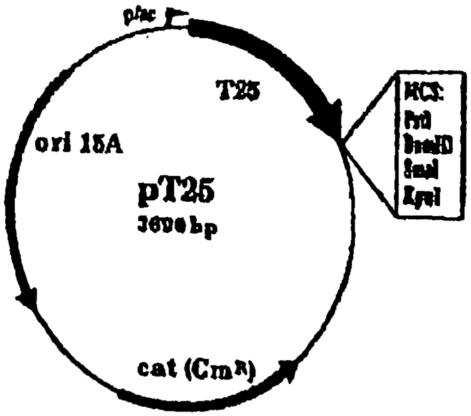
CAACGTCGTGACTGGGAAAACCCTGATCTATGAAT cgtagatactgaaaaacccc GCAA
162

GTT cacttcaactgtgcatcgtg caccatctcaatttcttt
ABS2 **53**

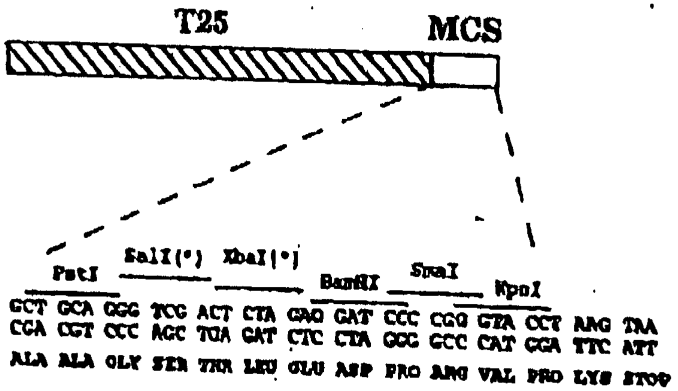
ABS1	**5' CGTTTGGAATCACTACAGG 3'**
JC90	**5' CGATGATGAAGATACCCCACCAAA 3'**
162	**5' GGGGTTTTTCAGTATCTACG 3'**
ABS2	**5' CACGATGCACAGTTGAAGTG 3'**
53	**5' GAAATTGAGATGGTGCACGATGCAC 3'**

FIGURE 11

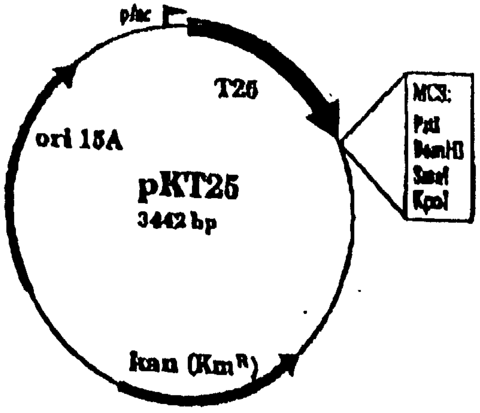
VECTORS EXPRESSING THE T25 FRAGMENT



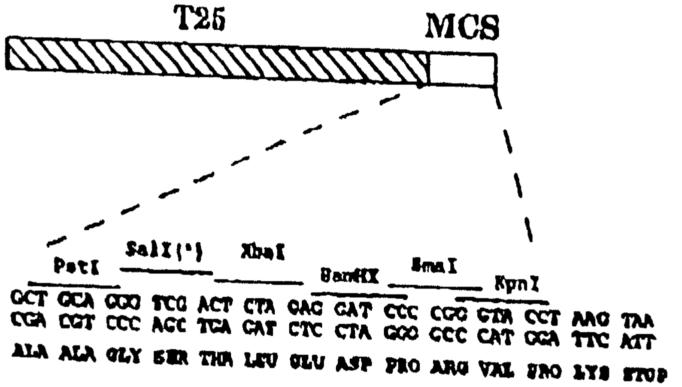
Derivative of pACYC184



(*) Restriction sites are not unique



Derivative of pSU40



(*) Restriction site is not unique

FIGURE 12

FIGURE 12

VECTORS EXPRESSING THE T18 FRAGMENT

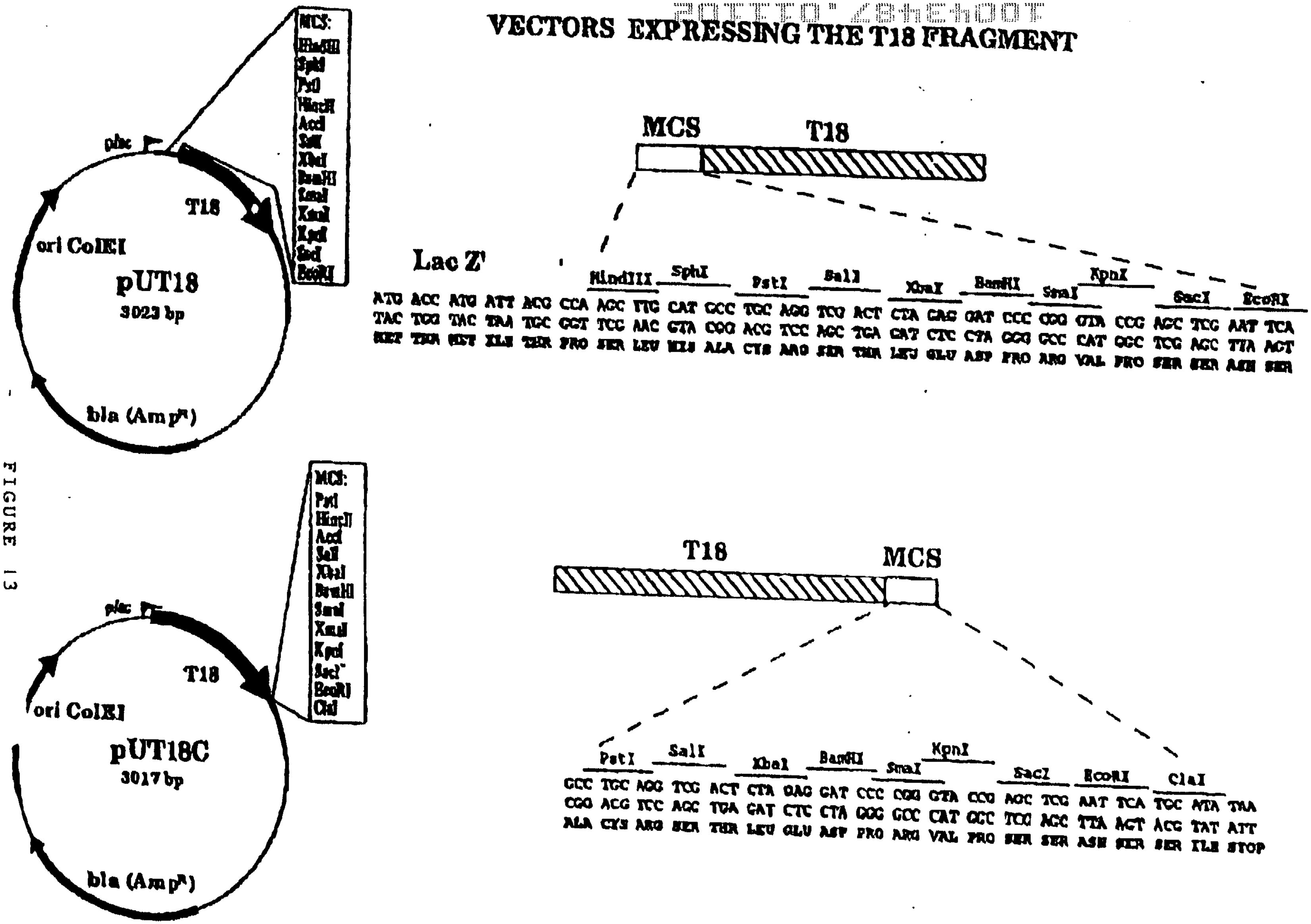


FIGURE 13

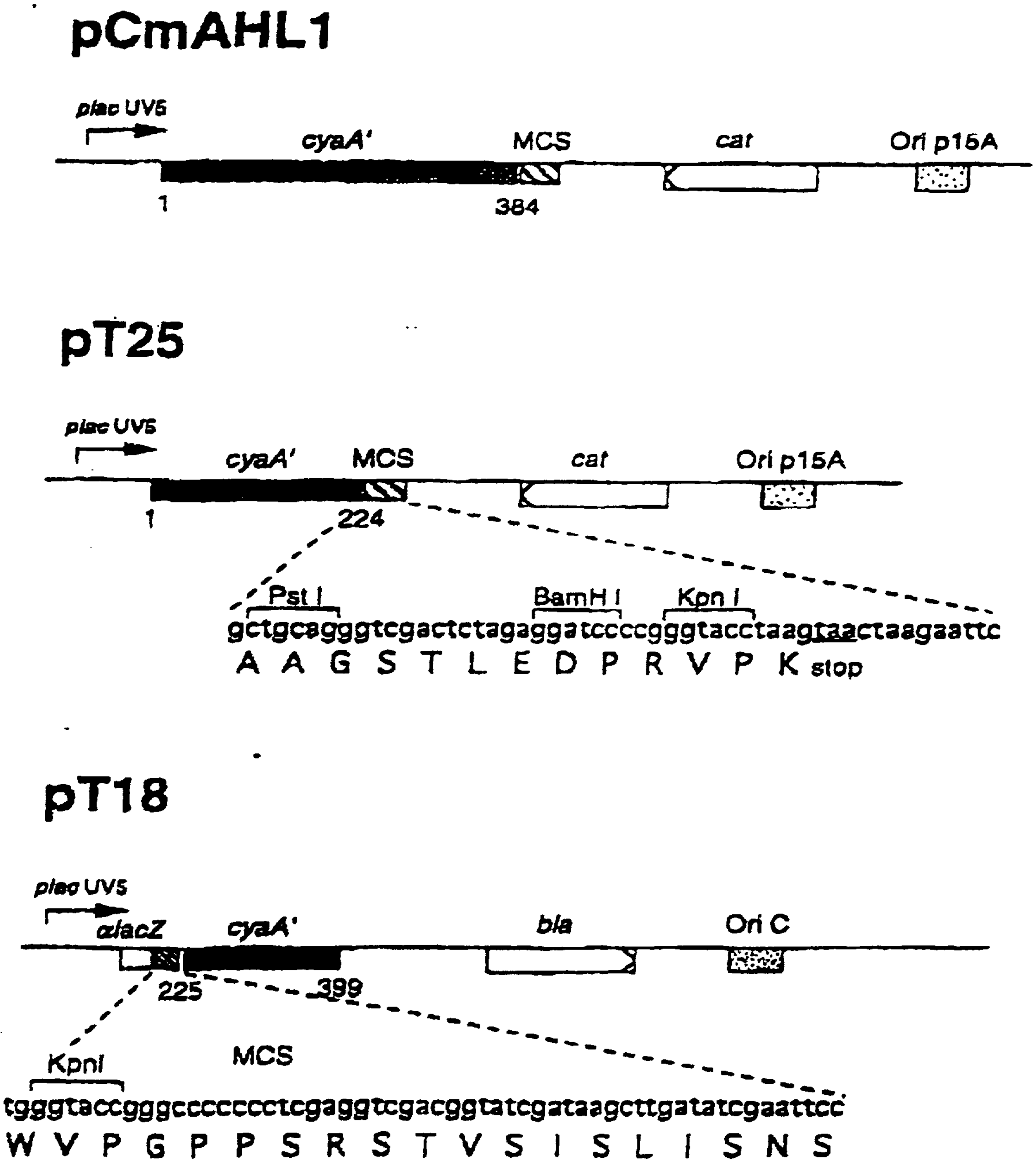

pCmAHL1
plac UV5
cyaA'
MCS
cat
Ori p15A
1
384
pT25
plac UV5
cyaA'
MCS
cat
Ori p15A
1
224
Pst I
BamH I
Kpn I
gctgcagggtcgactctagaggatccccgggtacctaagtaactaagaattc
A A G S T L E D P R V P K stop
pT18
plac UV5
αlacZ
cyaA'
bla
Ori C
225
399
KpnI
MCS
tgggtaccgggccccccctcgaggtcgacggtatcgataagcttgatatcgaattcc
W V P G P P S R S T V S I S L I S N S

FIGURE 14

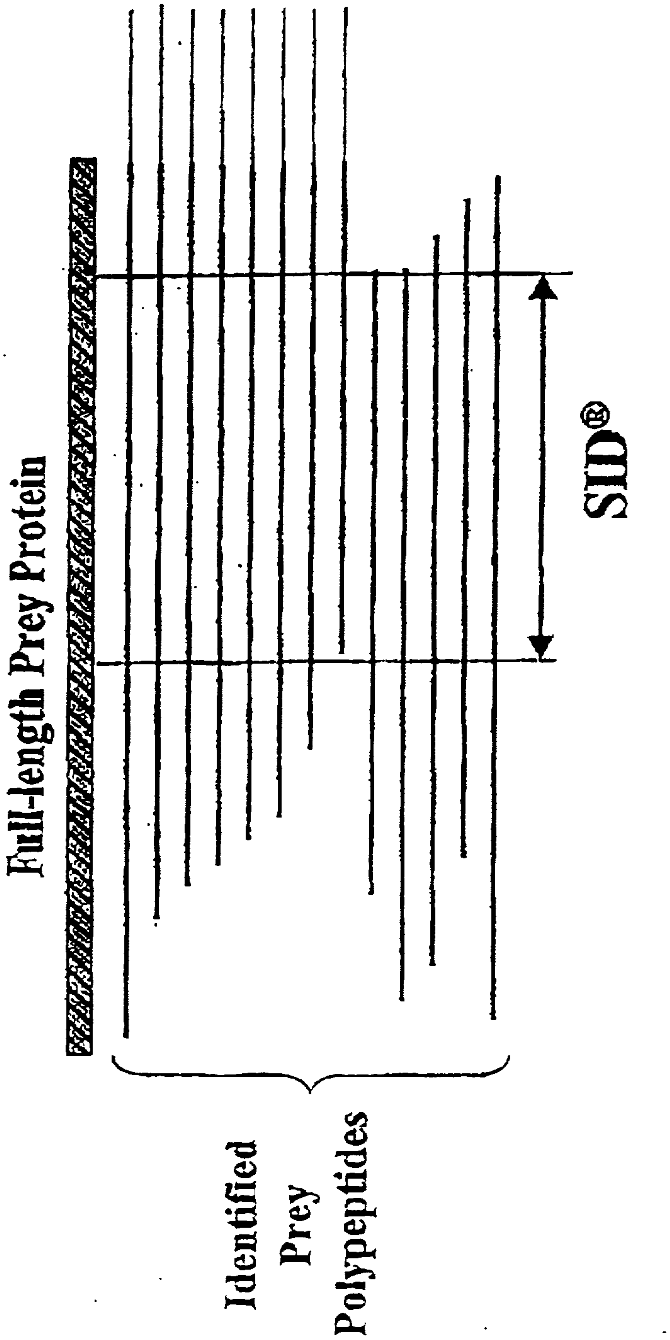


Figure 15 : schematic representation of SID® determination

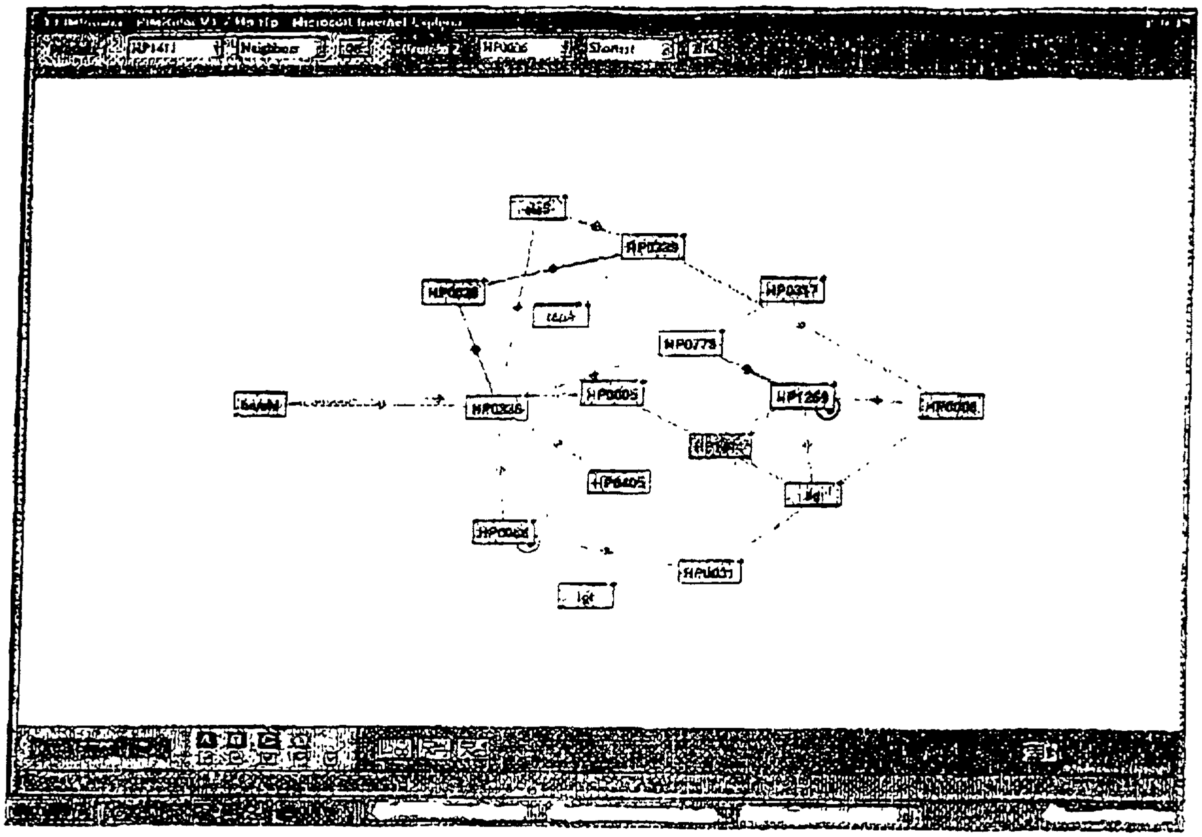


Figure 16 : Example of Protein Interaction Map